AD-A157 623 HEALTH HAZARD EVALUATION OF LIQUID MONOPROPELLANTS PHASE 4 SUBCHRONIC INH.. (U) ARMY ENVIRONMENTAL HYGIENE AGENCY ABERDEEN PROVING GROUND MD 19 JUL 85 1/1

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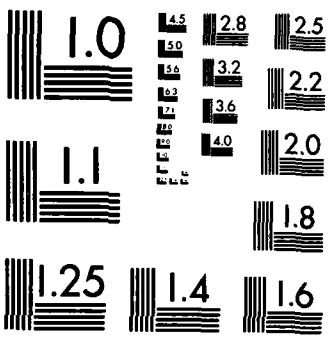

UNITED STATES ARMY ENVIRONMENTAL HYGIENE AGENCY

ABERDEEN PROVING GROUND, MD 21010-5422

PHASE 4
HEALTH HAZARD EVALUATION OF LIQUID MONOPROPELLANTS
STUDY NO. 75-51-0132-85
SUBCHRONIC INHALATION OF HYDROXYLAMMONIUM NITRATE
JANUARY 1985

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DEPARTMENT OF THE ARMY
U. S. ARMY ENVIRONMENTAL HYGIENE AGENCY
ABERDEEN PROVING GROUND, MARYLAND 21010-5422

Mr. Snodgrass/cvc/AUTOVON
584-3980

REPLY TO
ATTENTION OF

HSHB-OT

19 JUL 1985

SUBJECT: Phase 4, Health Hazard Evaluation of Liquid Monopropellants, Study No. 75-51-0132-85, Subchronic Inhalation of Hydroxylammonium Nitrate, January 1985

Commander
US Army Materiel Command
ATTN: AMCSG
5001 Eisenhower Avenue
Alexandria, VA 22333-0001

EXECUTIVE SUMMARY

The purpose, essential findings and major recommendations of the enclosed report follow:

a. Purpose. This study was conducted to determine the effects of repeated airborne exposures to animals of hydroxylammonium nitrate (HAN), a major component of liquid gun propellants. This evaluation will assist in advising on the potential health risks associated with handling these materials.

b. Essential Findings. Rats and dogs were exposed to aerosolized HAN for 90 days at concentrations of 300,100, and 33 mg/m^3. Dose-related effects occurred in both species and were characterized in rats by weight loss and spleen and liver enlargement. In dogs, respiratory irritation and blood dyscrasia were the major toxic effects. Minimal effects were observed at the low dose, 33 mg/m^3.

c. Major Recommendations. Personnel should be protected against all routes of HAN exposure since the systemic effects are additive. An airborne concentration of HAN at 3 mg/m^3 may be considered as a basis for the development of a maximum allowable workplace atmosphere. Accidental exposures in man should be closely monitored for cyanosis, anemia and respiratory distress. Treatment for methemoglobinemia may be indicated.

FOR THE COMMANDER:

Encl

JOEL C. GAYDOS
Colonel, MC
Director, Occupational and
Environmental Health

CF:
HQDA(DASG-PSP) (wo/encl)
Cdr, AMCCOM [AMSMC-SG(R)] (w/encl)
Cdr, ARRADCEN [DRSMC-MP-(A)] (w/encl)
Cdr, HSC (HSCL-P) (w/encl)
Comdt, AHS (HSHA-IPM) (w/encl)
Cdr, BRL (DRDAR-BLP) (w/encl)
Cdr, WRAMC (PVNTMED Svc) (w/encl)
Cdr, MEDDAC, Ft Meade (PVNTMED Svc) (2 cy) (w/encl)
C, USAEHA-Rgn Div North (w/encl)

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SUBCHRONIC INHALATION OF HYDROXYLAMMONIUM NITRATE
JANUARY 1985

1. AUTHORITY. Letter, US Army Ballistics Research Laboratory, DRDAR-BLP, 21 August 1978, subject: Request for Toxicity Tests on a Liquid Monopropellant, with indorsements thereto.

2. REFERENCES.

a. See Appendix A for a listing of references.

b. See Appendix B for a statement of quality assurance.

c. See Appendix C through J for tabular results.

3. PURPOSE. The purpose of this study was to determine the subchronic inhalation toxicity of hydroxylammonium nitrate (HAN) aerosol in dogs and rats exposed to the material for 13 weeks. This study was conducted to determine the relative inhalation toxicity, and therefore, to determine the possible health hazards associated with dermal or respiratory exposure.

4. BACKGROUND. Hydroxylammonium nitrate is the main constituent of several substances being considered for use as liquid gun propellants (LGP) by the US Army. Accidental aerosolization or spray of this material during manufacture or loading/filling operations poses a potential inhalation exposure situation. Previous studies performed by the Naval Medical Research Institute (Appendix A, reference 1) and the Navy Toxicology Unit (Appendix A, references 2 and 3) showed that similar compounds were moderately toxic to animals. Published reports (Appendix A, references 4 and 5) and current studies by this Agency show that HAN is a moderately toxic compound that acutely produces methemoglobinemia. The resultant respiratory distress and red blood cell destruction are its major toxic effects.

5. GENERAL. In the 90-day subchronic inhalation study, male and female rats and male Beagle dogs were exposed to aerosolized HAN 5 days a week for 13 weeks. The exposure period was 6 hours a day at airborne HAN concentrations of 300, 100, or 33 mg/m^3. Chamber control animals inhaled ambient room air only. Selected animals were tested for pulmonary function periodically throughout the study to assess changes in lung mechanics. Blood specimens were collected from dogs at 2-week intervals. Complete blood count (CBC), differential, and clinical chemistry measurements were performed. Animals were euthanized at the end of the 13-week exposure and necropsies performed. Additional groups of rats were sacrificed following 6 weeks of exposure, the study midpoint, and others at 10 weeks post-exposure.

6. MATERIALS AND METHODS.

a. Test Material. The HAN used in this study was provided by the US Army Ballistic Research Laboratory (USABRL), Aberdeen Proving Ground, Maryland. The material was supplied as a 13.24 molar aqueous solution containing approximately 80 percent HAN by weight. The chemical formula (NH_3OHNO_3) results in the molecular weight of 96. The compound has a density of 1560 mg/mL. It is a clear, odorless and somewhat viscous liquid extremely miscible in water. The material was identified as Naval Ordnance Station, Indian Head, Maryland, (NOSIH) Batch No. R149/151. The structure of HAN follows:

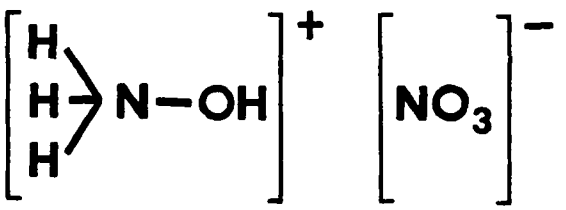

Figure. Hydroxylammonium Nitrate

b. Animal Usage *†. Two hundred forty Sprague-Dawley male and female rats initially weighing 100-125 gms were utilized in this study. They were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. The 16 male beagle dogs in the study were purchased from Laboratory Research Enterprises, Kalamazoo, Michigan. All animals were maintained on commercially prepared feeds. Food and water were available ad libitum except during the exposure periods. A 12-hour light-dark sequence was maintained. Ambient conditions were 24° C ± 2° and 45-55 percent relative humidity.

c. Subchronic Aerosol Exposure. The 13-week inhalation exposures were conducted using 1000 and 2000 liter dynamic flow chambers. The compound was held in glass reservoirs at ambient temperature and aerosolized using Collinson nebulizers with dried compressed air at pressures ranging from 20 to 40 psi depending on the concentration required. All chambers were sampled at 1, 3 and 5 hours daily to determine compound concentrations. The actual chamber concentrations were determined by drawing known volumes of chamber air through a glass bubbler system filled with a known quantity of deionized water. The amount of HAN trapped in the bubbler system was determined by measuring the electrical conductivity of the resulting solution with a Myron Model EP Conductivity Meter, Myron L Co., 6231 Yarrow Drive, Carlsbad, California. Readings were then compared to known standard dilutions of the material (Appendix A, reference 6).

d. Particle Size. Aerosol particle size was determined for each chamber using a cascade impactor at a fixed flow rate. Particle sizes were determined and are presented in paragraph 7b.

e. Clinical Signs. Animals were observed daily for signs of HAN toxicity. Body weights were measured and recorded for each animal biweekly. At necropsy, major organs were weighed and organ-to-body weight ratios determined.

f. Clinical Chemistry. Serum clinical chemistries were determined in dogs using an Abbott Bichromic Analyzer 100 (ABA-100)®. Sodium and potassium measurements were performed using an Instrumentation Laboratory

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* In conducting the studies described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," US Department of Health, Education and Welfare Publication No. (NIH) 78-23, revised 1978.

† The studies reported herein were performed in animal facilities fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

® Abbott Bichromatic Analyzer 100 (ABA-100) is a registered trademark of Abbot Diagnostics, South Pasadena, California.

Flame Photometer, Model 143, Instrumentation Laboratory Inc., Lexington, Massachusetts. Pretest measurements were made weekly, for 5 weeks. The following biochemical parameters were measured:

Serum glutamate oxaloacetic transaminase (SGOT)- Iu/l
Serum glutamic - pyruvic transaminase (SGPT) - Iu/l
α (alpha) - Hydroxybutyrate dehydrogenase (HBDH)- Iu/l
Creatine phosphokinase (CPK)- Iu/l
Lactic dehydrogenase (LDH)- Iu/l
Alkaline phosphatase (Alk Phos) - Iu/l
Gamma-glutamyl transferase (GGTP)- Iu/l
Blood urea nitrogen (BUN)- mg/dl
Glucose - mg/dl
Total protein - gm/dl
Total Bilirubin - mg/dl
Cholesterol - mg/dl
Triglyceride - mg/dl
Calcium (Ca) - mg/dl
Sodium (Na) - mEq/l
Potassium (K)- mEq/l

g. Hematology. Hematological measurements of dog blood were performed using a Coulter● Model ZBI6, automated blood counting system. The following measurements were made:

Total Erythrocyte Count (RBC)
Total Leukocyte Count (WBC)
Hematocrit (Hct)
Hemoglobin (Hgb)
Mean Corpuscular Volume (MCV)

A morphological evaluation of leukocytes (differential count) was also performed. Methemoglobin content of dog blood was determined by standard method (Appendix A, reference 7). Heinz body determinations were performed on whole dog blood using standard methods (Appendix A, reference 8).

h. Histopathology. Animals were euthanized at the completion of the 90-day exposure period and necropsied. Selected groups of rats were also necropsied at the study midpoint (6 weeks) and 10 weeks following the last exposure. Tissue specimens were harvested, fixed in 10-percent neutral formalin and later stained with hematoxylin and eosin for morphological examination. Histopathological evaluations were performed by commercial contractor (John W. Sargartz, DVM, Contract No. DAAD05-82-C-6037). The following tissue specimens from rats and dogs were evaluated:

Brain
Lungs
Heart
Liver
Stomach
Small intestine
Large intestine
Pancreas

● Coulter is a registered trademark of Coulter Electronics, Inc., Hialeah, Florida.

Kidney	Adrenal gland
Spleen	Urinary Bladder
Eye	Testes
Bone marrow	Ovaries
Bone	Skin
Trachea	Skeletal muscle
Nasal turbinates	Tongue
Thymus	Thyroid gland
Esophagus	Pituitary (dog)
Fat	Lymph node
	Spinal cord
	Peripheral nerve
	Mammary gland (rat)
	Salivary gland

i. Pulmonary Function. Changes in compliance and resistance functions of the lungs due to inhalation of airborne HAN were determined in all dogs. Specific procedures were earlier reported (Appendix A, reference 9). The following parameters were measured in each animal: air flow, tidal volume, intrapleural pressure, transpulmonary pressure, compliance and resistance. These parameters were measured or computed dynamically on a breath-to-breath basis and averaged every fifth breath. Pretest measurements were performed 1 week before the start of exposures. Posttest resistance and compliance values were determined within 1 week of the 90-day test completion.

7. RESULTS.

a. Subchronic Aerosol Exposures. The concentrations for the subchronic aerosol exposures were measured analytically as described in paragraph 6c. Table 1 is a list of the weekly averages of 6-hour time-weighted averages.

TABLE 1. WEEKLY AVERAGES OF THE DAILY TIME-WEIGHTED AVERAGES

Week No.	High Exposure Chambers (300 mg/m^3)		Middle Exposure Chamber (100 mg/m^3)	Low Exposure Chamber (33 mg/m^3)
	A	B	1	2
1	296	282	95	35
2	291	262	105	29
3	276	285	107	29
4	296	300	118	26
5	303	296	110	29
6	283	297	106	32
7	296	287	106	21
8	283	281	102	29
9	282	289	109	30
10	292	291	103	33
11	300	308	104	30
12	288	277	114	31
13	278	249	97	24

The mean and standard deviations of the daily time-weighted averages for the subchronic aerosols exposures are listed in Table 2.

TABLE 2. DAILY TIME-WEIGHTED AVERAGES FOR THE SUBCHRONIC AEROSOL STUDY

High Exposure Chambers (mg/m^3)		Middle Exposure Chamber (mg/m^3)	Low Exposure Chamber (mg/m^3)
A	B	1	2
290 ± 15	285 ± 28	107 ± 7	30 ± 4

b. Particle Characterization. Particle size distribution was determined for the aerosols generated in each chamber. Particle size analysis was accomplished using a Unico® Cascade Impactor, Model No. 216, at a flow rate of 0.50 SCFM. The test material was impacted on glass slides, washed from the slides with known volumes of reagent and the resultant concentrations assessed by measuring the conductivity of the solutions. Analysis of this data (Table 3) yielded results expressed as mass median diameter (MMD).

TABLE 3. ANALYSIS OF DATA EXPRESSED AS MMD

	High Exposure		Middle Exposure	Low Exposure
	Chamber A	Chamber B	Chamber 1	Chamber 2
MMD	1.04 μ	0.95 μ	1.04 μ	0.64 μ

c. Rat Clinical Signs. After the second day of HAN exposure, high dose rats were experiencing dyspnea and their breathing was audible. About 10 percent of the high dose rats died during their first week. The respiratory distress was rapidly resolved in the remaining animals and only the visible weight loss was noted thereafter. Additional deaths in high dose animals occurred sporadically during the last trimester of the study. By the end of the 90-day test, about 30 percent (10/35) of the high dose male rats had died and 35 percent of females (9/25). No compound-related deaths were observed in the middle or low dose animals or in the controls.

d. Rat Body Weights.

(1) Males. Inhaled HAN at the high exposure level was detrimental to the normal weight gain in rats. (See Appendix C.) A weight gain

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decrement (-36 g) versus control animals was observed by week 2 of exposure. By the end of the 13-week study, a -192 g difference was seen. When the exposures were stopped, rats at the high dose showed a rapid normalization in weight gain within 1 week and reached control limits by the 10th week. At the middle dose level, a similar but less pronounced decrement in weight gain was observed through 8 weeks of exposure. Beyond 8 weeks, no differences versus controls were noted. At the low exposure level, weight gains in rats were comparable with controls.

(2) Females. At the high dose level, female rats showed depressed weight gains by the second week of exposure compared to controls which persisted throughout the 13-week study. By test end, a 93 gm weight deficiency was recorded for high dose animals versus controls. A rapid weight gain occurred within 1 week following the completion of inhalation exposures and rat weights were within the control group range at 7 weeks postexposure. At the middle and low dose levels, body weights did not vary significantly from controls throughout the test and only marginal decrements were noted in the middle dose animals.

e. Rat Organ-to-Body Weight Ratio.

(1) Midterm. In both male and female rats sacrificed at the study mid-point (6 weeks of exposure), significant organ-to-body weight changes versus controls were observed. (See Appendices D and E.) At the high dose, rat spleen and heart ratios were elevated, also the testes in males. Spleen ratios were 3 to 4 times greater than controls. At the middle doses, spleen ratios were elevated in both sexes as were heart ratios in males. No significant variances were seen at the low dose.

(2) Term. At the end of the 90-day inhalation study in male and female rats, all organ-to-body weights ratios varied significantly from controls. All were elevated, particularly the spleen. In male rats, raw spleen weights were about 3 times heavier than measured in controls despite a 200 gm body weight deficit. At the middle dose, the spleen-to-body weight ratios were significantly higher in male and female rats compared to controls. Other affected organs in males were the kidneys and heart, both elevated. Low dose male rats had a slight elevation in organ-to-body weight ratios for heart, testes and spleen. A trend towards liver-to-body weight depression was noted in both male and female rats at the study end.

(3) Postexposure. By the tenth postexposure week, high dose male and female rats continued to demonstrate a significantly higher spleen-to-body weight ratio than controls. In females, the heart ratios also remained elevated. No significant variance from controls was observed in the middle and low dose rats 10 weeks after HAN exposure.

f. Dog Clinical Signs. After the first week of exposure, two of the four dogs at the high dose experienced anorexia, were dehydrated, and appeared debilitated. Mucous membranes were pale. A blood count was performed. Regenerative anemia, probably hemolytic, was diagnosed. After the second week, all of the dogs were anorexic. Dyspnea was observed and wheezing noted on auscultation. Tearing and a frothy, serous exudate from

the nostrils was seen in each high dose animal. Dogs at the middle dose also showed a mild, serous exudate from the nose and wheezing following 2 weeks of exposure. Near the end of 3-exposure weeks, all of the affected animals had regained their appetite and the inflammatory-type signs diminished. Only the serous nasal exudate remained in high dose animals. This appeared at the end of each exposure day and would disappear overnight. After 7 weeks of HAN exposure, high dose dogs again appeared dibilitated. On examination, mucous membranes were pale and nasal discharge persisted. The nares were hyperemic and on palpation, the spleens were enlarged. The animals improved rapidly and did not regress throughout the remainder of the study. Clinical signs in the middle dose animals disappeared after the first week of HAN exposure. No abnormal signs were observed in the low dose or control animals.

g. Dog Body Weight. A significant weight decrement (-1.3 kg) was observed in high dose dogs 2 weeks into the exposure which was gradually resolved through week 10. A second weight loss was noted in week 12 but interestingly, was also observed in dogs at the middle and low doses for that period. A weight decrement (-0.75 kg) was also observed in dogs at the middle exposure level at week 2 but was overcome by week 8 of the test. Dog weights at the low level were not significantly different than controls throughout the 13-week study. (See Appendix F.)

h. Dog Organ-to-Body Weight Ratio. Generally, no exposure-related differences in organ-to-body weight ratios were observed in dogs at any dose level. Only the kidneys from high dose dogs attained a significantly higher weight in relation to body mass when compared to control ratios. (See Appendix G.)

i. Dog Clinical Chemistry. Of the 16 clinical chemistry parameters measured, about half showed some significant variations from control values during the 13 weeks of exposure. (See Appendix H.) Many of these were transient and could not be related to inhaled HAN. The remainder, however, demonstrated either a dose-related response or some anomalous persistence during the test. A marked increase in LDH and a decrease in SGPT was observed within the first exposure week and persisted throughout the study. Both responses were dose-related. An increase in HBDH levels began at exposure week 1 at the high and middle doses and remained elevated. The BUN values were clearly depressed after the first week of exposure at all three dose levels but appeared normal thereafter. Interestingly, bilirubin levels were elevated only during the first weeks of exposure at the high dose. Generally, clinical chemistry values of low dose animals were unchanged throughout the 13-week test. Of the 16 parameters measured, only BUN, LDH and HBDH measurements varied from control limits sometime during the study. At test end, however, they were essentially normal.

j. Dog CBC Parameters. By the end of the first week of exposure dose-related CBC changes were apparent. (See Appendix I.) All parameters measured in dogs at the high exposure level were significantly different than controls. The RBC and Hct were markedly depressed while the WBC was elevated. Methemoglobin levels in the blood were elevated with an

accompanying Hgb depression. Heinz body formation involved about 94 percent of the circulating red cells of dogs at the high dose. At the middle exposure level (100 mg/m^3), similar CBC trends were observed but to a lesser degree. The WBC count was within the control range and Heinz body formation involved 34 percent of circulating RBC's. At the low exposure level (33 mg/m^3), a marginal WBC increase was observed during the first week when compared to control dog values. Beyond the first week of exposure, CBC parameters were not statistically different from control values at the low and medium dose levels except for Heinz body formation, which involved 32-48 percent of the RBC's in the middle dose dogs. This anomaly persisted throughout the study. Animals exposed at the high dose level also maintained formation of Heinz bodies in their blood, 90-95 percent, throughout the test. In addition, RBC, Hct, and Hgb levels remained depressed while methemoglobin was consistently higher. The WBC measurements, initially high, gradually declined to within control limits after the first week of the high dose exposure.

k. Dog Differential Count. An assessment of white blood cell morphology showed a marked increase in band neutrophils by the end of the first week of exposure at all levels. The response appeared dose-related. At week 5 of exposure, nucleated red blood cells predominated at the high level. At 100 mg/m^3, lymphocytes were depressed and segmented neutrophils were elevated. At the low level, no anomalies were observed. By the 9th week of exposure, nucleated red cells were still markedly elevated at the high dose level with an accompanying depression of lymphocytes. At the middle exposure level, an increase in the percent of segmented neutrophils versus controls was noted. A lymphocyte depression was also observed in the middle and low exposure blood specimens. Blood smears for the final (13 week) differential counts were lost. (See Appendix J.)

l. Histopathological Findings.

(1) In rats, the most significant microscopic lesion was hemosiderosis* in the spleen, liver, and kidney. This lesion was observed in high dose animals after 6 weeks of exposure, again at 13 weeks, and was still present 8 weeks postexposure. Hemosiderosis was seen at the middle dose level, only at the end of the 90-day study period. Splenic hemosiderosis was present in both control and treated groups; the difference was in severity rather than incidence. In comparison, kidney and liver hemosiderosis occurred only at the two high dose levels. Also attributed to the compound was dermatitis of the forepaw and rhinitis, seen only in the high dose group (300 mg/m^3). The remaining lesions were typical background changes frequently observed in the rat or those attributed to the method of euthanasia.

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* Hemosiderosis is a local or general increase in tissue iron stores without associated tissue damage.

(2) The increased size and weight of the rat spleen, observed at autopsy, was attributed to an increased amount of white pulp. It appeared that the number of RBC's in the sinusoids and macrophages containing hemosiderin increased with the exposure dose. Elevated liver and kidney weights are explained by the presence of hemosiderin in Kupffer cells and tubular epithelium.

(3) In dogs, hemosiderosis in the spleen and liver was the major compound related lesion seen at the end of the 90-day study. Glossitis and tracheitis and occasional bronchopneumonia were also observed. The incidence of lesions was clearly dose-dependent except in the spleen. In that organ, severity rather than incidence was evaluated since controls were also affected.

(4) The hemosiderosis in both species suggests the presence of abnormal RBC morphology, with removal of red cells from the circulation. Blood measurements in dogs support this hypothesis, i.e., dose-related decreases in Hct, RBC's, and Hgb. In addition, no evidence of compound-related hemorrhage was observed grossly or microscopically in the rat or dog.

m. Pulmonary Function. Table 4 shows the pre and posttest values for compliance and resistance in treated dogs. The mean values in the high and middle dose groups were not different ($P < 0.05$) from pretest controls. At the low dose, posttest resistance values were significantly different compared with controls. This was not considered compound related since pretest resistance values in low dose animals also varied significantly from controls.

TABLE 4. SUMMARY OF COMPLIANCE AND RESISTANCE VALUES IN DOGS EXPOSED TO HAN (Mean Value ± SD)

Treatment Group	Pretest Compliance	Posttest Compliance	Pretest Resistance	Posttest Resistance
	mL/ cm H_2O/ kg			
Control	6.54 ±0.56	5.16 ±0.46	0.04 ±0.02	0.05 ±0.00
300 mg/m^3	6.55 ±0.79	4.75 ±1.26	0.04 ±0.03	0.05 ±0.01
100 mg/m^3	6.68 ±0.48	5.08 ±2.08	0.04 ±0.03	0.06 ±0.03
33 mg/m^3	6.63 ±0.54	4.26 ±0.66	0.16* ±0.02	0.06* ±0.01

* Significantly different from control at $p < 0.05$ (Mann-Whitney U-test)

8. DISCUSSION.

a. Our studies have demonstrated dose-dependent systemic toxic effects in animals following repeated inhalation exposures to aerosolized HAN. At the lowest airborne concentration, 33 mg/m^3, only minimal effects are observed in dogs and rats. The effects of the two higher concentrations, 100 and 300 mg/m^3, were characterized in rats by continual weight decrements, marked organ-to-body weight ratio changes, and deaths (high dose only). The body weight decrements were reversed in surviving rats during the 60-day recovery period but the enlarged spleens of high dose animals remained unresolved. In dogs, blood dyscrasia was the dominant effect of repeated HAN exposures. This was accompanied by upper respiratory irritation, a direct chemical effect. Interestingly, dogs appeared to adapt to the continued HAN insult after the first week of exposure with regards to the measured blood effects. A gradual rebound towards pretest control values was observed during the remaining 12 weeks.

b. Aerosolized HAN, when inhaled, apparently reacts with the mucoid lining of the respiratory tract causing inflammation. This was characterized in the dog by glossitis, tracheitis and occasional bronchopneumonia. Similar observations were made in rats but could not be unequivocally attributed to HAN because of the high incidence of comparable lesions seen in controls. The airborne HAN particles, averaging about 1.0 micron in diameter, would be expected to be deposited into the smaller airways and alveoli of the lungs. (Appendix A, reference 10).

c. The sequelae of absorbed HAN following inhalation exposure was consistent with that observed after dermal, oral, or parenteral treatment. (Appendix A, references 4 and 5). Once HAN reaches the systemic circulation, it acts directly on the red blood cells (erythrocytes) by oxidizing Hgb and producing methemogoblin. These changes are evidenced by the rapid formation of Heinz bodies (proteinaceous clumps) within the erythrocytes and an accompanying decrement in the oxygen-carrying capacity of blood. Imperfect erythrocytes are removed within the sinusoids of the liver and spleen. The resulting congestion may explain the splenic enlargement seen in rats. Also, the body's attempt to conserve and recycle the heme (iron) portion of the oxidized Hgb results in the accumulation of iron within the liver and spleen. This condition is described pathologically as hemosiderosis. Other effects of erythrocyte depletion include weight loss and an increase in heart size and weight.

d. In animals, a sustained airborne concentration of HAN at 33 mg/m^3 was noninjurious following long-term exposure. Similar observations would be expected in man. Incorporating a tenfold safety margin, an atmospheric HAN concentration of 3-4 mg/m^3 would be considered a basis for the development of a workplace standard. Short-term exposures (15 minutes or less) at 300 mg/m^3 would not be deemed incapacitating as demonstrated in dogs and permanent injury would not be anticipated. In each case, the concommitant additive effects of skin exposure to HAN must be considered.

9. RECOMMENDATIONS.

a. Maximize protection against inhalation, oral, and dermal exposures to HAN; and include protective gloves, aprons, splash guards, and respirators as appropriate to good industrial hygiene practice.

b. Recommend that an airborne concentration of HAN at 3 mg/m^3 be considered as a basis for the development of a maximum allowable workplace atmosphere. Caution towards the additive effects of other exposure routes must be exercised (paragraph 8d, this report).

c. Closely monitor accidental exposures in man for symptoms of respiratory distress and the development of cyanosis. Treatment for methemoglobinemia may be indicated. (This recommendation is based on the results shown in Appendices C through J.)

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APPENDIX A

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2. Letter, US Navy Toxicology Unit, National Naval Medical Center, 31 August 1970, subject: Monopropellants; toxicological evaluation of, with indorsements thereto.

3. Letter, US Navy Toxicology Unit, National Naval Medical Center, 11 January 1971, subject: NOS 283 Monopropellant; toxicological evaluation of.

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6. "Standard Methods for the Examination of Water and Wastewater", 14th ed., American Public Health Assn., Washington D.C., pp 71-73.

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APPENDIX B

ANALYTICAL QUALITY ASSURANCE

The Analytical Quality Assurance Office certifies the following:

a. These studies were conducted in accordance with:

(1) Standing Operating Procedures developed by the Toxicology Division, USAEHA.

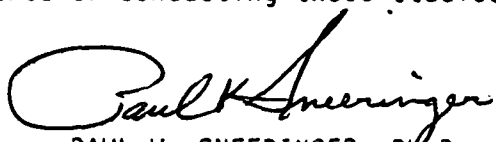
(2) Title 21, Code of Federal Regulations (CFR), 1983 rev, Part 58, Good Laboratory Practice for Nonclinical Laboratory Studies.

(3) Final Rule, Pesticide Programs; Good Laboratory Practice Standards; 48 Federal Register (FR) 53946-53969, 29 November 1983.

(4) Final Rule, Toxic Substances Control; Good Laboratory Practice Standards; 48 Federal Register (FR) 53922-53944, 29 November 1983.

b. Facilities were inspected during its operational phase to ensure compliance with paragraph a above.

c. The information presented in this report accurately reflects the raw data generated during the course of conducting these studies.



PAUL V. SNEERINGER, PH.D.
Chief, Analytical Quality
Assurance Office

APPENDIX C

HAN SUBCHRONIC INHALATION, RAT BODY WEIGHTS (gm)

	Week	Control	Exposure Level 300 mg/m³	100 mg/m³	33 mg/m³
Male	0	243±14	243±15	243±16	244±18
	2	296±22	259±19	280±25	300±23
	4	339±27	258±28	320±24	345±27
	6	378±36	256±40	362±30	382±32
	8	410±44	250±48	384±35	398±34
	10	442±50	256±57	419±40	431±37
	12	457±53	266±53	435±43	447±40
Postweek	1	458±53	336±35	465±39	461±30
Postweek	7	512±60	453±58	541±42	520±29
Postweek	10	495±86	502±51	574±47	545±29
Female	0	164±11	167±14	164±12	169±9
	2	198±16	179±13	192±15	201±11
	4	221±18	184±15	214±15	225±13
	6	238±20	194±17	233±17	243±14
	8	248±23	184±21	236±17	246±19
	10	262±26	178±23	251±16	262±20
	12	267±24	173±28	258±19	266±20
Postweek	1	272±27	233±12	257±15	284±16
Postweek	7	298±30	264±3	274±34	317±29
Postweek	10	306±41	276±3	289±40	327±30

APPENDIX D

HAN SUBCHRONIC INHALATION, RAT ORGAN-TO-BODY WEIGHT RATIO (MALES)

Group	Organ	Exposure Level Control	300 mg/m³	100 mg/m³	33 mg/m³
6-week Sacrifice	BDY WT (gm)	406±14	294±35	388±24	490±28
(Midterm)	Liver	3.54±0.28	3.17±0.41	3.37±0.22	3.19±0.25
	Spleen	0.19±0.02	0.70±0.14	0.31±0.05	0.21±0.02
	Heart	0.29±0.02	0.40±0.03	0.33±0.02	0.33±0.04
	Kidney	0.73±0.07	0.82±0.08	0.74±0.04	0.76±0.04
	Testes	0.74±0.07	1.04±0.15	0.85±0.09	0.77±0.13
13-week Sacrifice	BDY WT (gm)	485±56	254±44	460±43	481±47
(Term)	Liver	3.11±0.26	3.57±0.67	3.09±0.23	2.90±0.28
	Spleen	0.16±0.02	0.92±0.14	0.26±0.04	0.17±0.02
	Heart	0.27±0.02	0.53±0.14	0.29±0.02	0.31±0.06
	Kidney	0.64±0.06	0.83±0.06	0.70±0.04	0.66±0.07
	Testes	0.68±0.09	0.89±0.23	0.73±0.08	0.74±0.07
	Brain	0.42±0.05	0.73±0.11	0.45±0.04	0.43±0.04
Postweek 10 Sacrifice	BDY WT (gm)	495±86	502±51	574±48	545±29
	Liver	4.06±0.55	3.78±0.25	3.54±0.29	3.65±0.21
	Spleen	0.16±0.04	0.26±0.02	0.16±0.04	0.15±0.02
	Heart	0.36±0.11	0.29±0.03	0.28±0.30	0.28±0.05
	Kidney	0.75±0.19	0.70±0.06	0.66±0.05	0.66±0.05
	Testes	0.68±0.14	0.72±0.06	0.55±0.09	0.62±0.05
	Brain	0.44±0.08	0.42±0.04	0.37±0.04	0.38±0.02

APPENDIX E

HAN SUBCHRONIC INHALATION RAT ORGAN-TO-BODY WEIGHT RATIO (FEMALES)

Group	Organ	Exposure Level Control	300 mg/m³	100 mg/m³	33 mg/m³
6-week Sacrifice	BDY WT (gm)	269±54	192±29	233±21	273±54
(Midterm)	Liver	3.26±0.37	3.72±0.67	3.47±0.35	3.03±0.38
	Spleen	0.21±0.05	0.73±0.21	0.34±0.04	0.22±0.02
	Heart	0.30±0.04	0.42±0.07	0.36±0.05	0.30±0.04
	Kidney	0.73±0.12	0.85±0.12	0.85±0.11	0.66±0.09
13-week Sacrifice	BDY WT (gm)	283±32	178±25	273±19	269±20
(Term)	Liver	3.36±0.20	4.40±0.59	3.43±0.29	3.08±0.43
	Spleen	0.20±0.04	1.09±0.46	0.31±0.03	0.21±0.03
	Heart	0.32±0.04	0.59±0.08	0.34±0.03	0.34±0.03
	Kidney	0.73±0.08	1.02±0.14	0.68±0.20	0.77±0.08
	Brain	0.67±0.06	0.99±0.13	0.71±0.04	0.67±0.09
Postweek10 Sacrifice	BDY WT (gm)	306±41	276±2	289±39	327±30
	Liver	3.64±0.40	3.67±0.43	3.58±0.33	3.62±0.28
	Spleen	0.19±0.04	0.30±0.05	0.20±0.02	0.17±0.02
	Heart	0.30±0.02	0.35±0.01	0.32±0.02	0.36±0.09
	Kidney	0.73±0.05	0.69±0.05	0.74±0.05	0.72±0.06
	Brain	0.64±0.09	0.69±0.05	0.66±0.08	0.60±0.05

APPENDIX F

HAN SUBCHRONIC INHALATION STUDY, DOG BODY WEIGHTS (kg)

MEAN OF FOUR ANIMALS

Level	Pretreat -2	Week +2	Week +4	Week +6	Week +8	Week +10	Week +12	Week +13
Control	10.98 ±0.70	11.33 ±0.90	10.98 ±0.92	11.55 ±0.75	11.80 ±0.71	11.95 ±0.73	11.22 ±0.78	11.52 ±0.88
300 mg/m^3	10.95 ±0.81	9.68* ±0.90	9.72* 0.74	10.32* ±0.87	10.60* ±0.95	10.65* ±1.11	9.50* ±0.83	10.30* 0.62
100 mg/m^3	10.98 ±0.97	10.22* ±0.76	10.10* ±0.90	10.55* ±0.90	10.76 ±0.50	10.88 ±0.51	10.18 ±0.55	10.75 ±0.54
33 mg/m^3	10.78 ±1.19	11.15 ±1.23	11.02 ±1.31	11.38 ±1.22	11.75 ±1.25	12.05 ±1.29	11.10 ±1.14	11.55 ±1.18

* Significantly different body weight gain versus controls

APPENDIX G

HAN SUBCHRONIC INHALATION STUDY, ORGAN-TO-BODY WEIGHT RATIOS (DOGS)

MEAN OF FOUR ANIMALS

Level	Body Weight (kg)	Organ-to-body Weight Ratio					
		Brain	Liver	Spleen	Heart	Kidney	Testes
Control	11.53 ±0.88	0.74 ±0.14	3.32 ±0.35	0.58 ±0.25	0.82 ±0.07	0.48 ±0.04	0.10 ±0.05
300 mg/m^3	10.30 ±0.62	0.76 ±0.03	3.66 ±0.33	1.31 ±0.62	0.96 ±0.06	0.60* ±0.07	0.16 ±0.03
100 mg/m^3	10.75 ±0.54	0.76 ±0.05	3.42 ±0.24	0.76 ±0.16	0.88 ±0.15	0.54 ±0.05	0.16 ±0.04
33 mg/m^3	11.55 ±1.18	0.73 ±0.06	3.00 ±0.23	0.56 ±0.17	0.89 ±0.04	0.54 ±0.05	0.12 ±0.03

* Significantly different compared to control ratios

APPENDIX H

HAN SUBCHRONIC INHALATION, DOG CLINICAL CHEMISTRY MEASUREMENTS

Level	Test*	Pretest	Week 1	Week 5	Week 9	Postweek 1
Control	SGPT	47±4	53±9	54±14	50±7	65±18
	HBDH	77±29	59±38	59±9	34±10	58±12
	LDH	82±34	42±22	43±5	32±7	61±10
	BUN	15±3	23±4	25±9	28±6	19±4
	Bilirubin	0.5±0.1	0.4±0.1	0.6±0.2	0.8±0.1	0.6±0.0
300 mg/m^3	SGPT	55±17	27±5	21±2	22±4	25±6
	HBDH	76±27	162±77	214±104	96±28	98±17
	LDH	83±36	169±88	240±130	113±54	96±12
	BUN	15±3	13±2	18±7	18±5	18±3
	Bilirubin	0.5±0.0	0.9±0.2	0.7±0.1	0.9±1.0	0.8±0.1
100 mg/m^3	SGPT	46±5	38±8	41±5	37±1	35±7
	HBDH	76±13	128±34	174±32	104±60	142±18
	LDH	93±26	132±39	194±48	132±82	145±10
	BUN	17±4	14±3	19±2	26±6	26±5
	Bilirubin	0.6±0.1	0.5±0.1	0.6±0.1	0.9±0.2	0.8±0.1
33 mg/m^3	SGPT	44±8	43±4	54±11	48±7	52±6
	HBDH	83±19	48±12	68±9	46±14	97±22
	LDH	92±31	42±14	60±13	54±22	105±30
	BUN	16±3	16±2	26±7	24±4	18±2
	Bilirubin	0.5±0.1	0.4±0.0	0.8±0.2	0.9±0.1	0.6±0.1

* Presentation of results of transient or nonresponses has been omitted. These included SGOT, CPK, ALk Phos, GGTP, Glucose, Protein, Cholesterol, Ca, Triglyercides, Na and K.

APPENDIX I

HAN SUBCHRONIC INHALATION, DOG CBC AND HEINZ BODIES MEASUREMENTS

MEAN OF FOUR ANIMALS/GROUP

Level	Test	Pretest	Week 1(2)	Week 5(6)	Week 9	Week 13
Control	RBC	6.68	6.24	6.39	6.14	6.49
	Hct	43.25	39.96	44.38	41.40	43.60
	MCV	65.00	64.25	69.00	66.50	67.00
	WBC	15.25	14.92	14.62	13.78	12.95
	Hgb	16.28	14.98	15.18	15.32	15.75
	met Hgb	--	(0.00)	(0.00)	0.00	0.00
	Heinz B.	0.00	0.00(0.00)	(0.00)	0.00	0.00
300 mg/m^3	RBC	6.27	2.90	3.87	3.88	4.20
	Hct	41.87	21.35	31.75	30.60	33.15
	MCV	67.00	74.50	82.00	80.00	79.25
	WBC	13.50	47.02	25.52	26.90	18.20
	Hgb	15.87	8.42	10.18	11.08	11.42
	met Hgb	--	(8.30)	(14.80)	8.40	7.10
	Heinz B.	0.00	93.75(95.00)	(90.00)	--	60.00
100 mg/m^3	RBC	6.88	4.16	5.82	5.08	5.80
	Hct	44.60	28.58	42.15	36.38	41.72
	MCV	65.00	69.25	72.50	71.25	71.50
	WBC	13.70	18.58	15.18	14.20	25.25
	Hgb	17.08	10.20	13.65	13.55	14.78
	met Hgb	--	(0.00)	(0.00)	0.00	0.00
	Heinz B.	0.00	33.75(47.50)	(32.50)	--	42.50
33 mg/m^3	RBC	6.58	6.12	6.08	5.91	6.58
	Hct	43.75	41.28	44.02	42.25	46.82
	MCV	66.00	67.50	71.75	70.75	70.75
	WBC	10.55	12.05	10.88	11.52	10.95
	Hgb	16.38	14.90	14.75	15.42	16.32
	met Hgb	--	(0.00)	(0.00)	0.00	0.00
	Heinz B.	0.00	0.00(0.00)	(0.00)	0.00	0.00

APPENDIX J

HAN SUBCHRONIC INHALATION, DOG DIFFERENTIAL COUNT
MEAN OF FOUR DOGS/GROUP

Level	Cell Type	Pretest	Week+1	Week+5	Week+9
Control	Lymph	44.3±23.2	28.0±10.0	35.5±5.1	31.5±4.4
	S Neut	43.3±25.1	62.0±12.8	57.2±8.0	58.2±6.1
	Mono	5.3±2.1	6.5±1.7	1.5±2.4	5.0±3.7
	Eosin	6.3±4.5	3.0±3.2	5.0±4.9	4.0±4.3
	Baso	0.0±0.0	0.0±0.0	0.0±0.0	0.5±1.0
	B Neut	0.7±0.6	0.5±0.6	0.8±1.0	0.8±1.5
	NRBC	1.0±1.7	2.2±1.0	1.5±1.3	3.0±0.8
300 mg/m^3	Lymph	49.3±17.7	22.0±11.6	28.5±9.5	19.0±3.9*
	S Neut	43.7±15.9	55.2±15.0	61.0±9.4	67.5±7.1
	Mono	2.3±2.3	9.2±1.5	6.5±2.6*	7.2±5.9
	Eosin	3.7±0.6	2.0±2.2	2.8±1.0	0.5±0.6
	Baso	0.3±0.6	0.0±0.0	0.0±0.0	1.5±1.0
	B Neut	0.7±0.6	12.2±7.1*	1.2±1.0	4.2±1.0*
	NRBC	0.0±0.0	9.0±8.5	24.0±14.2*	14.0±5.8*
100 mg/m^3	Lymph	37.7±29.1	18.2±4.8	17.8±2.2*	18.0±0.8*
	S Neut	56.3±29.7	67.8±6.1	74.5±5.3*	71.5±2.4*
	Mono	4.3±1.2	7.2±1.5	2.5±1.7	4.8±4.2
	Eosin	1.3±2.3	2.5±1.7	3.8±4.1	2.8±3.1
	Baso	0.0±0.0	0.0±0.0	0.5±1.0	0.5±0.6
	B Neut	0.3±0.6	4.5±2.1*	0.8±0.5	2.5±1.7
	NRBC	0.7±1.2	6.2±3.9	2.2±1.7	5.5±4.2
33 mg/m^3	Lymph	52.5±22.8	28.0±1.8	27.8±4.0	23.0±3.9
	S Neut	40.2±21.2	58.5±3.8	64.8±1.5	66.2±3.3
	Mono	5.2±1.7	5.0±1.4	1.8±2.4	5.2±1.9
	Eosin	2.0±1.8	5.0±4.8	4.8±2.2	3.8±2.1
	Baso	0.0±0.0	0.5±0.6	0.2±0.5	1.0±0.8
	B Neut	0.0±0.0	3.0±0.8*	0.8±1.5	0.8±1.0
	NRBC	0.2±0.5	2.0±1.6	2.5±2.4	4.0±2.2

* Significantly different from controls

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